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Copper-containing DNA–Silica Mineral Complexes for the Asymmetric Diels–Alder Reaction

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In this study, we have generated DNA–silica minerals containing a Cu(ligand) complex and evaluated their utility in asymmetric synthesis. Cu(dmbpy)/DNA–silica mineral could be applied successfully to the Diels–Alder reaction and reused readily for 10 cycles without loss of enantioselectivity.

DNA, the ubiquitous biopolymer that exists in all living organisms on earth, is nowadays exploited in a wide range of research fields from biology as genetic information storage materials to materials science as a useful building block. DNA has received attention as an attractive chiral source for asymmetric synthesis because of its unique helical chirality, chemical stability, and water solubility.[1–3] In 2005, Feringa and Roelfes introduced a new concept of biohybrid catalysis based on catalytically active metal complexes, binding ligands, and DNA, the so-called DNA-based hybrid catalysts.[4] Since then, DNA-based asymmetric catalysis has been recognized and actively studied as an environmentally friendly strategy for the synthesis of enantiomerically pure compounds.[5–20] Our group is exploring the potential of DNA for asymmetric synthesis. We have developed asymmetric intramolecular Friedel–Crafts alkylations using a self-assembled DNA hybrid catalyst.[21,22] To understand the structural and mechanistic features of DNA-based asymmetric catalysis, we have devised a systematic DNA hybrid catalyst based on the direct incorporation of an intrastrand bipyridine ligand into the DNA phosphate backbone and demonstrated its application to the asymmetric intramolecular Friedel–Crafts alkylations.[23] We have also focused on a heterogeneous reaction system, which has a number of practical benefits, such as the easy separation of products/catalysts by filtration and recyclability of catalysts. In 2013, we devised a method to immobilize DNA on a silica support based on the electrostatic interaction between its anionic phosphate backbone and cationic quaternary ammonium-functionalized silica, and demonstrated that this supported DNA could be reused as a chiral source in the Cu(II)-catalyzed Diels–Alder reaction in water.[24] This is the first example of a recyclable solid-supported DNA applied to asymmetric catalysis. Benedetti *et al.* reported that a cellulose-supported DNA-based hybrid catalyst could catalyze Friedel–Crafts alkylations and Michael additions with good-to-high enantioselectivities.[25] They also demonstrated continuous-flow processes using their cellulose-supported DNA-based hybrid catalyst. Despite this notable advance, heterogeneous DNA hybrid catalysts have rarely been employed for asymmetric synthesis. Considering the high industrial relevance of heterogeneous catalysis, merging DNA-based asymmetric synthesis and heterogeneous catalysis needs further investigation.[26–29] Che *et al.* developed DNA–silica complexes by DNA self-assembly and silica mineralization using *N*-trimethoxysilylpropyl-*N,N,N*-trimethyl ammonium chloride (TMAPS) as a costructure directing agent, cationic Mg(II) ions, and a silica source tetraethoxysilane (TEOS).[30–37] These studies prompted us to investigate the utility of DNA–silica minerals for DNA-based asymmetric synthesis. To our knowledge, metal-containing DNA–silica minerals have not yet been explored as heterogeneous DNA-based hybrid catalysts. Herein, we report that Cu(dmbpy)/DNA–silica minerals can catalyze the asymmetric Diels–Alder reactions[39–43] with high conversion, excellent *endo/exo* selectivities, and high enantioselectivities up to 99% *ee*.

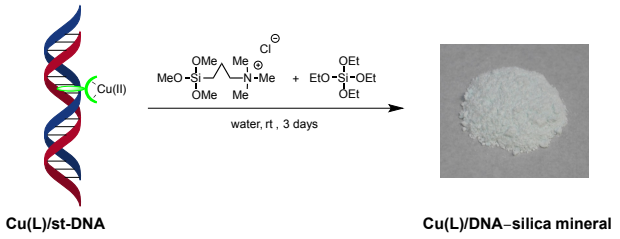


Figure 1. Preparation of metal(ligand)-containing DNA–silica minerals. The picture shows Cu(dmbpy)/DNA–silica minerals.

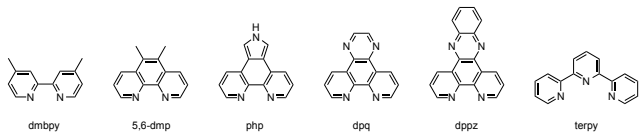
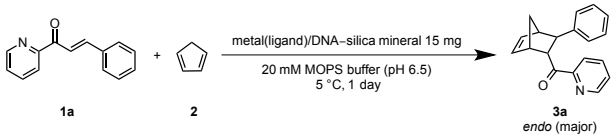


Figure 2. Biding ligands for copper-containing DNA–silica minerals

We first prepared standard Mg/DNA–silica mineral by following the procedures established by Che *et al*., in which salmon testes DNA (st-DNA) was used as a readily available DNA source (see SI). To examine the utility of DNA–silica minerals in DNA-based asymmetric synthesis, we performed Cu(II)-catalyzed asymmetric Diels–Alder reactions with aza-chalcone (**1a**) and cyclopentadiene (**2**) in the presence of 15 mg of DNA–silica mineral in 20 mM MOPS buffer solution (300 μl, pH 6.5) at 5 °C for 1 day.[24,38] The enantioselectivity and conversion were determined by HPLC analysis based on the literature.

Table 1. Asymmetric Diels–Alder reactions with aza-chalcone **1a** and cyclopentadiene **2** in the presence of metal/DNA–silica minerals.



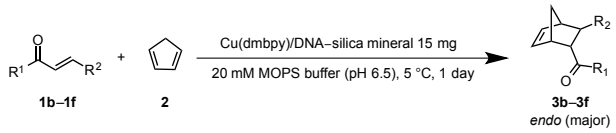
1a **2** **3a** *endo* (major)

entry	metal complex	enantioselectivity (% *ee*)[a]	conversion (%)[a]
1	$MgCl_2$	–	trace
2[b]	$MgCl_2$	84	68
3	$Cu(NO_3)_2$	10	79
4	Cu(dmbpy)	99	97
5	Cu(5,6-dmp)	45	35
6	Cu(php)	19	49
7	Cu(dpq)	13[c]	84
8	Cu(dppz)	12	66
9	Cu(terpy)	9[c]	23

[a]Experiments were conducted using 1 μmol of aza-chalcone, 24 μmol of cyclopentadiene, and 15 mg of Cu(L, L = ligand)/DNA–silica mineral at 5 °C in 20 mM MOPS buffer (pH 6.5) for 1 day unless otherwise noted. The conversion, *endo*/*exo* selectivities, and enantioselectivities were determined by chiral HPLC analysis. All of the reactions gave *endo*/*exo* selectivities higher than 96%. Therefore, the enantioselectivities in this paper are only for the *endo* isomer. Results represent the average value of more than two experiments. [b]Preincubated reaction suspension 15 mol% of Cu(dmbpy) with 15 mg of Mg/DNA–silica mineral in 20 mM MOPS buffer solution (300 μl, pH 6.5) at 5 °C for 1 h. [c] (–)-enantiomer of Diels-Alder product was obtained.

A reaction with only Mg/DNA–silica mineral showed almost no conversion. Interestingly, when we used preincubated reaction suspension that was prepared by mixing 15 mol% of Cu(dmbpy) with 15 mg of Mg/DNA–silica mineral in 20 mM MOPS buffer solution (300 μl, pH 6.5) at 5 °C for 1 h, the Diels–Alder adduct was obtained in moderate conversion and good enantioselectivity (68% conversion and 84% *ee*, entry 2 in Table 1). This promising result prompted us to investigate DNA–silica mineralization using Cu(L)-containing DNA complexes in place of Mg(II) ions. We generated DNA–silica complexes with a variety of Cu(L) complexes as follows: 1.6 mM of *N*-trimethoxysilylpropyl-*N*,*N*,*N*-trimethylammonium chloride (TMAPS) and 4.3 mM of tetraethyl orthosilicate (TEOS) were added to the prepared Cu(L)/DNA solution and mixed for 30 min. Then, the solution was left for 3 days at RT. As the Cu(L) complexes used for the mineralization, various pale-colored powders were obtained and examined in the Cu(II)-catalyzed asymmetric Diels–Alder reactions as shown in Table 1. The standard reaction conditions were determined as follows: 1 μmol aza-chalcone **1a** (0.5 M solution in acetonitrile) and 24 μmol cyclopentadiene **2** were added to 20 mM MOPS buffer solution (300 μl, pH 6.5) including 15 mg of Cu(L)/DNA–silica mineral and mixed by continuous rotation at 5 °C for 1 day. Cu(II)/DNA–silica mineral without a bidentate ligand gave the product in low enantioselectivity (entry 3 in Table 1). To our delight, when we performed the reaction with Cu(dmbpy)/DNA–silica mineral, Diels–Alder product was obtained with excellent enantioselectivity (up to 99% *ee*), almost full conversion (97%), and good reproducibility (entry 4 in Table 1, Table S1). As a result, we found that different enantioselectivities and conversions of Diels–Alder adducts were observed depending on the Cu(L) complexes comprising the DNA–silica mineral. Although a Cu(dmbpy)/DNA–silica mineral afforded the highest enantioselectivity of the product as previously established homogeneous DNA-based Cu(II)-catalyzed Diels–Alder reactions,[44] a general tendency between the Cu(L)/DNA–silica minerals and the observed enantioselectivities was not consistent with the results by homogeneous DNA-based Cu(II)-catalyzed Diels–Alder reactions. This suggests that the chiral microenvironment in heterogeneous Cu(L)/DNA–silica minerals might be different from that in homogeneous Cu(L)/DNA duplexes in water. We also cannot exclude the possibility that various chiral environments surrounding DNA and inside DNA affect the outcome of the reactions.[45]

Table 2. Investigation of substrate scope for the asymmetric Diels–Alder reaction.



1b–1f **2** **3b–3f** *endo* (major)

1b : R^1 = 2-pyridyl, R^2 = *o*-ClC_6H_4
1c : R^1 = 2-pyridyl, R^2 = *p*-BrC_6H_4
1d : R^1 = 2-pyridyl, R^2 = *p*-$MeOC_6H_4$
1e : R^1 = 2-pyridyl, R^2 = *p*-$NO_2C_6H_4$
1f : R^1 = 2-(1-methylimidazolyl), R^2 = C_6H_5

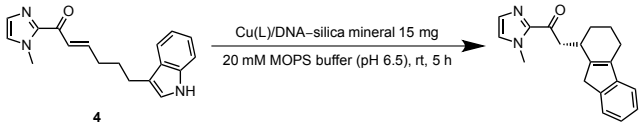
entry	products	*ee* (%)[a]	conversion (%)[a]
1	**3b**	85	97
2[b]	**3c**	86	45
3[b]	**3d**	35	95
4	**3e**	99	99
5	**3f**	81	97

[a]Experiments were conducted using 1 μmol of aza-chalcone, 24 μmol of cyclopentadiene, and 15 mg of Cu(dmbpy)/DNA–silica minerals at 5 °C in 20 mM MOPS buffer (pH 6.5) for 1 day unless otherwise noted. The conversion, *endo*/*exo* selectivities, and enantioselectivities were determined by chiral HPLC analysis. All of the reactions gave *endo*/*exo* selectivities higher than 96%. Therefore, the enantioselectivities in this paper are only for the *endo* isomer. Results represent the average value of more than two experiments. [b]The reactions were performed at RT.

Based on the results from Table 1, the scope of aza-chalcones, **1b**–**1f**, was investigated under standard reaction conditions. Aza-chalcones having *o*-chlorophenyl group (**1b**) and *p*-nitrophenyl group (**1d**) gave the desired Diels–Alder products with high enantioselectivity (85% and 99% *ee*, respectively) and almost full conversion (entries 1 and 4 in Table 2). The substituents on the aza-chalcone such as *p*-bromophenyl (**1c**) and *p*-methoxyphenyl group (**1d**) required ambient temperature to increase the conversion of substrates. Unfortunately, in the case of aza-chalcones having *p*-methoxyphenyl group (**1b**), a significant decrease of

enantioselectivity was observed (35% *ee*, entry 3, Table 2). Regarding the 2-acyl pyridyl moiety on aza-chalcone, the 2-acyl-*N*-methylimidazoyl moiety could replace the 2-acyl pyridyl group and afforded the desired product with 81% *ee* and 97% conversion (entry 5, Table 2). When we performed the reaction with chalcone instead of aza-chalcone, almost no conversion was observed. The bidentate substrates are crucial for the reaction progress.

Table 3. Intramolecular Friedel–Crafts alkylation using Cu(L)/DNA–silica minerals.

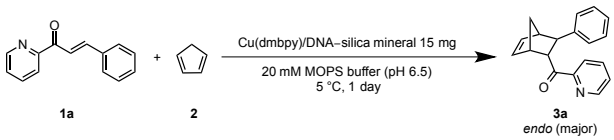


ligand	dmbpy	5,6-dmp	php	dpq	dppz	terpy
ee (%)[a]	43	69	18	37	50	13
yield (%)[a]	22	25	31	16	9	4

[a]Experiments were conducted using 1 μmol tethered indole substrate and 15 mg Cu(L)/DNA–silica minerals at RT in 20 mM MOPS buffer (pH 6.5) for 5 h unless otherwise noted. The yields and enantioselectivities were determined by chiral HPLC analysis. Results represent the average value of more than two experiments.

To verify the usability of Cu(L)/DNA–silica minerals, intramolecular Friedel–Crafts alkylations were conducted with various Cu(L)/DNA–silica minerals (Table 3). The best result (69% *ee*) was obtained with Cu(5,6-dmp)/DNA–silica mineral and this is comparable to the previous result from homogeneous DNA-based intramolecular Friedel–Crafts alkylation.[23] Like the Diels–Alder reactions, different enantioselectivities of the products were obtained depending on the Cu(L) complexes comprising DNA–silica minerals. Although the yields of the product were generally low, these results demonstrate the potential application for Cu(L)/DNA–silica minerals for DNA-based asymmetric synthesis. Considering the amount of Cu(dmbpy) complex and availability of Cu(dmbpy)/DNA–silica minerals, we performed Cu(II)-catalyzed Diels–Alder reaction at preparative scale. Aza-chalcone **1a** (50.2 mg; 0.24 mmol) and cyclopentadiene **2** (5.6 mmol) were added to 20 mM MOPS buffer solution (20 ml, pH 6.5) in the presence of 70 mg Cu(dmbpy)/DNA–silica mineral containing less than 7 mol% of Cu(dmbpy) complex at 5 °C. After 3 days, 50.0 mg of Diels–Alder adduct was obtained as a mixture of the *endo* and *exo* isomers (*endo*/*exo* = 32:1) in 76% yield with 91% *ee* for the *endo* isomer (Figure S2). This result clearly indicates that Cu(dmbpy)/DNA–silica minerals can be used as catalysts for the asymmetric Diels–Alder reaction. Furthermore, by taking the advantage of a heterogeneous catalyst, we examined the reusability of Cu(dmbpy)/DNA–silica mineral in the asymmetric Diels–Alder reaction. After the reaction followed by simple extraction, Cu-dmbpy/DNA–silica mineral was recovered and reused for the next reaction with fresh MOPS buffer solution (20 mM, pH 6.5). Table 4 shows the experimental results for the recyclability of Cu-dmbpy/DNA–silica mineral. Under standard reaction conditions, Cu(dmbpy)/DNA–silica mineral could be recycled up to 10 times and very high enantioselectivity >97% was obtained in every cycle.

Table 4. Investigation of recyclability of Cu(dmbpy)/DNA–silica mineral in asymmetric Diels–Alder reaction.



cycles	1	2	3	4	5	6	7	8	9	10
ee (%)[a]	99	98	99	99	99	99	99	98	97	98
conversion (%)[a]	97	85	89	79	92	91	82	80	80	77

[a]Experiments were conducted using 1 μmol aza-chalcone, 24 μmol cyclopentadiene, and 15 mg Cu(dmbpy)/DNA–silica minerals at 5 °C in 20 mM MOPS buffer (pH 6.5) for 1 day unless otherwise noted. The conversion, *endo*/*exo* selectivities, and enantioselectivities were determined by chiral HPLC analysis. All of the reactions gave *endo*/*exo* selectivities higher than 96%. Therefore, the enantioselectivities in this paper are only for the *endo* isomer. Results represent the average value of more than two experiments.

In conclusion, we have prepared Cu(dmbpy)/DNA–silica minerals and successfully applied them in the Cu(II)-catalyzed asymmetric Diels–Alder reactions with excellent enantioselectivity and almost full conversion (up to 99% *ee* and 99% conversion). A variety of DNA–silica minerals containing Cu(ligand) complexes could be generated using a straightforward method and they are stable in air and water. In addition, Cu(dmbpy)/DNA–silica mineral was readily reusable up to 10 cycles without loss of enantioselectivity. Although further study is needed to clarify the chiral induction mechanism of Cu(L)/DNA–silica mineral, this proof of concept study shows that metal-containing DNA–silica minerals can serve as durable heterogeneous biohybrid catalysts for asymmetric synthesis. We are currently developing new DNA–silica minerals using various metal complexes besides Mg(II) ions and Cu(II) ions for DNA-based asymmetric synthesis.

Supporting Information is available on http://dx.doi.org/10.1246/cl.******.

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Conflict of interest statement. None declared.

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45 Previously, Che and co-workers have reported that the helicity of the DNA-silica complexes can be controlled by cationic Mg(II) ion and TMAPS concentration. In this study, we only focused the bidentate ligands for Cu(II) complex in conjunction with the enantioselectivities of the desired products. We are under study to investigate the effect of Cu(L) complexes on the helicity of Cu(L)/DNA-silica minerals.

NOTE *The diagram is acceptable in a colored form. Publication of the colored G.A. is free of charge.*
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If the data of your G.A. is "bit-mapped image" data (not "vector data"), note that its print-resolution should be 300 dpi.
You are requested to put a brief abstract (50-60words, one paragraph style) with the graphical abstract you provided, so that readers can easily understand what the graphic shows.

Graphical Abstract

Textual Information

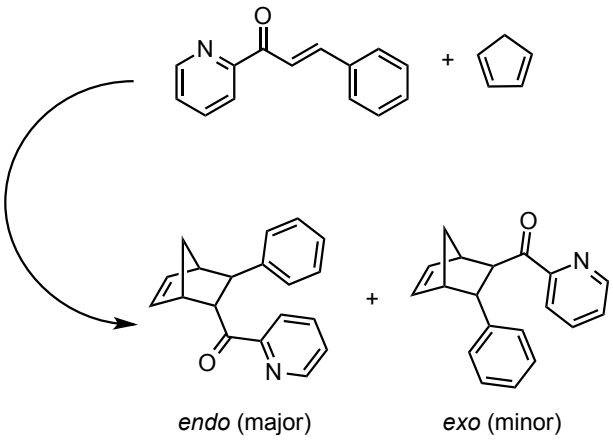
A brief abstract (required)	In this study, we have generated DNA–silica minerals containing a Cu(ligand) complex and evaluated their utility in asymmetric synthesis. Cu(dmbpy)/DNA–silica mineral could be applied successfully to the Diels–Alder reaction and reused readily for 10 cycles without loss of enantioselectivity.
Title(required)	**Copper-containing DNA–Silica Mineral Complexes for the Asymmetric Diels–Alder Reaction**
Authors' Names(required)	Sohei Sakashita,[1] Soyoung Park,*[1] and Hiroshi Sugiyama*[1,2]

Graphical Information

Cu(dmbpy)/DNA-silica mineral

easy to prepare

stable & recyclable



endo (major) *exo* (minor)

up to 97% conversion
endo / *exo* > 40 : 1
up to 99% *ee* for *endo*

Supporting Information

Copper-containing DNA–Silica Mineral Complexes for the Asymmetric Diels–Alder Reaction

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Materials

Deoxynucleic Acid from salmon testes (st-DNA) was purchased from Aldrich and used as received. *N*-trimethoxysilylpropyl-*N*,*N*,*N*-trimethylammonium chloride (TMAPS) and tetraethyl orthosilicate (TEOS) were purchased from TCI and used as received. All other chemicals and solvents were purchased from Sigma-Aldrich Chemicals Co., Wako Pure Chemical Ind. Ltd., TCI, and Nacalai Chemical Co. Water was deionized (specific resistance of > 18.0 MW cm at 25 °C) by a Milli-Q system (Millipore Corp.). Aza-chalcone **1a** was synthesized as reported procedure.[1] Cyclopentadiene was prepared by cracking dicyclopentadiene. Substrate **4** for intramolecular Friedel-Crafts alkylations was synthesized as previously reported.[4]

Methods

For the synthesis of substrates and the preparative-scale reactions, NMR spectra were obtained on a JEOL JNM ECA-600 spectrometer operating at 600 MHz for ^{1}H NMR and in $CDCl_3$ unless otherwise noted. Flash Column chromatography was performed employing WakoGel

60N (63–212 mesh, Wako). Silica-gel preparative thin-layer chromatography (PTLC) was performed using plates from Silica gel 70 PF254 (Wako Pure Chemical Ind. Ltd.). Enantiomeric excess (ee) was determined by HPLC analysis (Chiralcel OD-H, AD-H) using UV-detection. Detail HPLC conditions were shown in the previous study.[2] DNA concentration was measured by Nanodrop ND-1000 spectrophotometer. Rotary mixing of the reaction suspension was performed by Intelli-Mixer RM-2 (Elmi). Eyela FDU-1100 was used for the freeze-drying.

Preparation of Cu(ligand)/DNA–silica minerals

43.2 mg of st-DNA was solved in the 33 ml of deionized water. To st-DNA solution, 0.023 mmol of (20 mM Cu(ligand) solution, 840 μl) was added. After mixing well, 180 μl of *N*-trimethoxysilylpropyl-*N*,*N*,*N*-trimethylammonium chloride (TMAPS), 300 μl of tetraethyl orthosilicate (TEOS) were added to the solution. After the mixing for 1 h at 5 °C, the solution was left to stand at ambient temperature for 3 days. Cu(ligand)/DNA–silica mineral was collected by centrifugation (13,000 rpm for 4 min), then washed by 10 ml of deionized water at 3 times, and freeze-dried. In this procedure, 60–100 mg of the Cu(ligand)/DNA–silica minerals was usually obtained as the powder.

General Procedure for the Diels-Alder reactions using Cu(ligand)/DNA–silica minerals

The reaction was conducted in 1.5 ml of eppendorf tube. 15 mg of Cu(ligand)/DNA–silica mineral was added to 300 μl of 20 mM MOPS buffer (pH 6.5), and mixed by continuous rotation at 5 °C for 1 h. After 1 h, 1 μmol of aza-chalcone **1a** (0.5 M solution in acetonitrile) and 24 μmol of cyclopentadiene **2** (2 μl) were added to the solution and mixed by continuous rotation at 5 °C for 1 day. The products were extracted with diethyl ether at 3 times and the solvent was removed under reduced pressure. The e.e. of the product was determined on a Daicel Chiralcel OD-H column with a solvent mixture, hexane: 2-propanol = 95:5, under a flow rate of 0.5 mL/min. The HPLC conditions of other substrates were determined as the previously reported study.[1–4]

Investigation of DNA leaching from Mg/ DNA–silica mineral

15 mg of Mg/DNA–silica mineral was added to the 300 μl of 20 mM MOPS buffer (pH 6.5) in 1.5 ml of eppendorf tube. Then suspension was set to rotary mixer and mixed by continuous rotation at 5 °C. In order to monitor DNA concentration by leaching from Mg/DNA–silica mineral, we measured the absorbance intensity at 260 nm in supernatant liquid after the centrifugation (13,000 rpm for 1min).

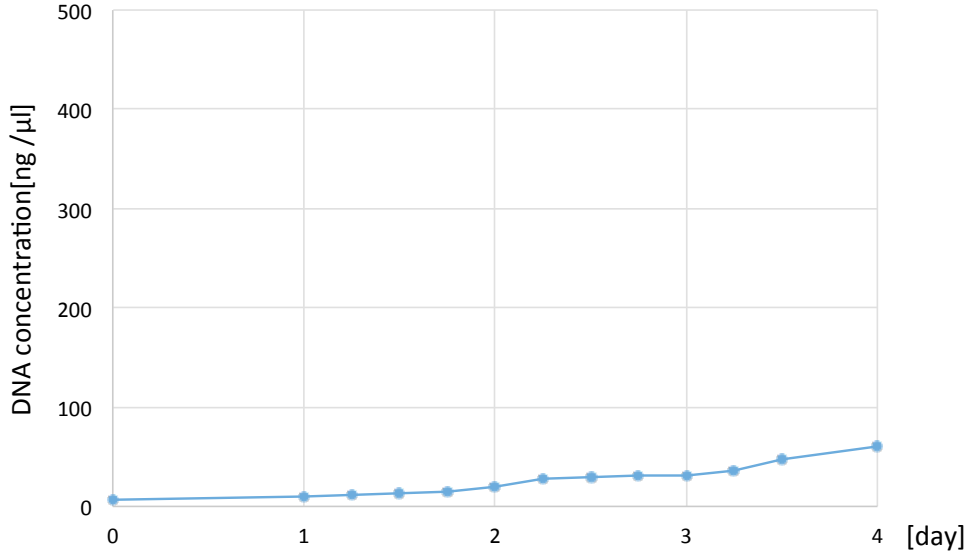


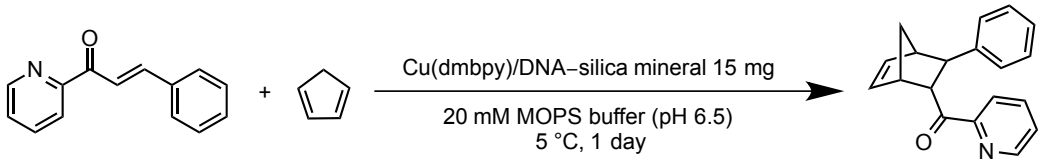
Figure S1. DNA concentration in MOPS buffer solution in the presence of Mg/DNA–silica mineral.

The blue dots represent DNA concentration in MOPS buffer solution in the presence of Mg/DNA–silica mineral. At the beginning of the experiment, the absorbance intensity at 260 nm in supernatant liquid indicated 6.0 ng/μl of DNA concentration. After 1day mixing, the concentration of DNA concentration was observed up to 9.7 ng/μl. Based on this result, we

conducted the Diels-Alder reaction using 9.7 ng/μl st-DNA solution containing 3 mol% of Cu(dmbpy). As a result, the desired product was obtained in 28% *ee* and low conversion.

Reproducibility of Cu(dmbpy)/DNA–silica mineral

For the reproducibility investigation, 5 samples of Cu(dmbpy)/DNA–silica minerals were generated independently and examined in the Diels-Alder reaction. The reactions were conducted using 1 μmol aza-chalcone, 24 μmol cyclopentadiene, and 15 mg Cu(dmbpy)/DNA–silica minerals at 5 °C in 20 mM MOPS buffer (pH 6.5) for 1 day. As shown in the Table S1, every Cu(dmbpy)/DNA–silica mineral afforded the desired product with excellent enantioselectivities and high conversions.

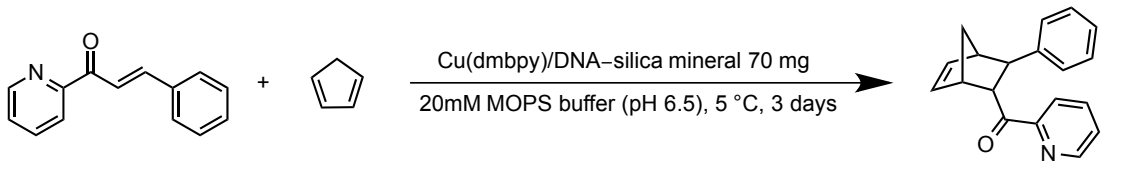


1a **2** **3a** *endo* (major)

lot number	1	2	3	4	5	6	7	8	9	10	average value
ee (%)[a]	94	92	98	87	93	92	94	97	93	99	95 ± 2.5
conversion (%)[a]	96	98	96	86	88	85	91	87	95	95	92 ± 4.9

Table S1. Reproducibility of Cu(dmbpy)/DNA–silica mineral

Preparative scale Diels-Alder reaction



1a (0.24 mmol) **2** (5.6 mmol) less than 7 mol% of Cu(dmbpy) **3a** 76% yield *endo*/*exo* = 32/1 91% *ee* for *endo* isomer

Figure S2. Preparative scale Diels-Alder reaction with Cu(dmbpy)/DNA–silica mineral

The reaction was conducted in 50 ml of conical tube. 70 mg of Cu(ligand)/DNA–silica mineral was added to 20 ml of 20 mM MOPS buffer (pH 6.5), and mixed by continuous rotation at 5 °C for 1 h. After 1 h, 0.24 mmol of aza-chalcone **1a** (0.8 M solution in acetonitrile) and 5.8 mmol of cyclopentadiene **2** were added to the solution and mixed by continuous rotation at 5 °C for 3 days. The reaction mixture was extracted with diethyl ether at 3 times and the solvent was

removed under reduced pressure. The residue was purified by silica gel preparative TLC with Hexane/EtOAc = 3:1 to afford the compound **3a** as a colorless oil (50.0 mg, 76% yield, *endo*/*exo* = 32:1, 91% *ee* for *endo* isomer). The *ee* and *endo*/*exo* ratio of the product were determined on a Daicel Chiralcel OD-H column with a solvent mixture, hexane:2-propanol = 95:5, under a flow rate of 0.5 mL/min.

Procedure for reusability of Cu(dmbpy)/DNA–silica mineral

The Diels-Alder reactions were conducted as above-mentioned in general procedure. After the extraction with diethyl ether, Cu(dmbpy)/DNA–silica mineral was recovered by centrifugation and reused for the next reaction with 300 μl of fresh MOPS buffer solution (20 mM, pH 6.5).

References

(1) G. Roelfes, and B. L. Feringa. *Angew. Chem., Int. Ed.*, **2005**, *44*, 3230.

(2) S. Park, K. Ikehata, H. Sugiyama. *Biomater. Sci.*, **2013**, *1*, 1034.

(3) S. Park, I. Okamura, S. Sakashita, S. J. H. Yum, A. Chiranjit, L. Gao, H. Sugiyama. *ACS Catalysis*, **2015**, *5*, 4708.

(4) S. Park, K. Ikehata, R. Watabe, Y. Hidaka, A. Rajendran, H. Sugiyama. *Chem. Commun.*, **2012**, *48*, 10398.